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Association of Neamine and its Derivative with the Ribosomal A-Site RNA

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With Brownian dynamics (BD) we study the mechanism and kinetics of association of neamine, an aminoglycosidic antibiotic, and its proposed derivative with the ribosomal A-site RNA. We compare the performance of different antitibiotic models used during BD simulations.

1 Introduction

The aminoacyl-tRNA site (A-site) in the 30S bacterial ribosomal subunit is the binding site for most aminoglycosidic antibiotics[1]. Binding of aminoglycosides interferes with translation and causes the decrease of its fidelity[2]. Although used in medical therapy, antibiotics from this family suffer from moderate affinity, inadequate specificity and may cause damage to mammalian and kidney cells. Also, bacterial resistance limits their effectiveness in medical therapy. Therefore, different theoretical[3–6] and experimental[3,7,8] approaches are applied in order to understand their binding mechanism and efforts to improve their selectivity and efficiency are being made. We believe that for aminoglycosides' inhibitory role, it is not only important how strong are the bound complexes (which is usually one of the criteria applied during computer-aided drug design process) but also how fast they can be formed. Therefore, we applied the BD technique to examine first stages of binding of the neamine and designed by us model compound (Figure 1). Neamine derivative was proposed during a two stage screening procedure which utilized a pharmacophoric search for possible binding modes, followed by a binding affinity estimation (Piotr Setny, unpublished results). The antibiotic target used in BD simulations is a symmetric RNA oligonucleotide containing two ribosomal A-sites[9] (Figure 1). In our recent work[4] we studied association of various aminoglycosides with this RNA fragment. In this study improved models of antibiotics are used in an attempt to validate previous results.

2 Methods

2.1 Brownian Dynamics Methodology

BD allows one to simulate the diffusional motion between interacting solutes and compute diffusion limited rate constants of their association. The ligand is represented as a polymer composed of spherically symmetric subunits, with centrally assigned partial charges, diffusing through the electrostatic field generated by a receptor. Ligand's motion can be derived using Ermak-McCammon[10] propagation scheme:

$$r_i^{n+1} = r_i^n + \sum_j \frac{\Delta t}{k_B T} D_{ij}^n F_j^n + R_i(\Delta t) \qquad (1)$$

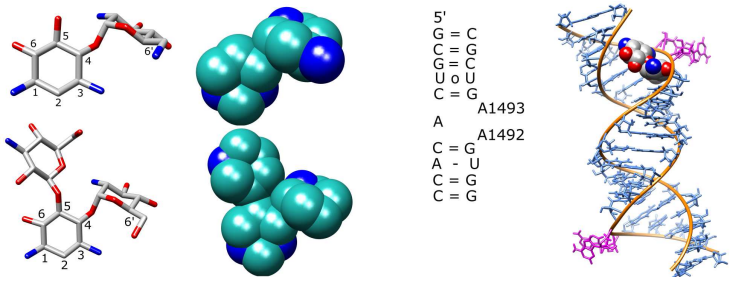

Figure 1. Left: Atomic structures and bead models used in BD of neamine (top) and its derivative (bottom). Blue beads correspond to amine groups and bear charges $+1$e. Green beads are neutral. Right: A-site RNA duplex. Secondary structure of the asymmetric unit (containing one A-site). Adenines 1492 and 1493 (*E. Coli* numbering) are displaced upon binding of an antibiotic[2]. Base pairs are represented with $=$ and $-$ (corresponding to three or two hydrogen bonds, respectively) for Watson-Crick pairs and o for non-Watson-Crick pairs. Three dimensional structure of the RNA duplex (blue and orange) with the A-site occupied by neamine (shown as van der Waals spheres); A1492 and A1493 are denoted in magenta.

where indices i and j run over coordinates of N subunits $(1 \leq i, j \leq 3N)$, r_i is the position vector component, F_i is the sum of intersubunit and external forces acting in direction i, integer n represents discrete times $t = n\Delta t$ at intervals Δt, D_{ij} is the diffusion tensor, and $R_i(\Delta t)$ is a random vector whose average is zero and $< R_i(\Delta t)R_j(\Delta t) >= 2D_{ij}^n\Delta t$. In BD, a ligand moves in the potential generated by fixed, rigid acceptor, and obtained from the solution of the Poisson-Boltzmann equation[11] on the 3D grid. External forces acting on the ligand are computed as a sum of exclusion and electrostatic terms. To estimate association rates, a large number of BD trajectories is generated. Each trajectory begins with the ligand placed randomly on the surface of the sphere with radius b. In a simulation, the ligand either diffuses outside the sphere with radius q (where $q >> b$) and the trajectory is truncated or forms an encounter complex with the receptor, satisfying some predefined reaction criteria. The ratio of the number of reactive trajectories to their total number allows one to estimate the association rate constant[12].

2.2 Brownian Dynamics Simulations Setup

The coordinates of the symmetric RNA fragment (Figure 1) with two paromomycins bound at A-sites were taken from the Protein Data Bank (code 1J7T[9]). Paromomycin coordinates were used as a template for positioning the neamine and model compound within the A-site and to establish reaction criteria defining the encounter complex. We represented neamine with 13 and its derivative with 18 spherical subunits (beads). Beads were centered on atoms of antibiotic's rings (Figure 1). Aminoglycosides were shown to bind to RNA in their fully protonated state[13] thus we assigned a total net charge of $+4$e to both molecules (distributions of partial charges are shown in Figure 1). Ligand and RNA hydrodynamic parameters were derived from their all-atom structures using the procedure described previously[4]. Resulting translational diffusion coefficients are $4.1 \cdot 10^{-6} \frac{cm^2}{s}$ for neamine and $3.5 \cdot 10^{-6} \frac{cm^2}{s}$ for its derivative. Electrostatic calculations and BD simulations utilized the University of Houston Brownian Dynamics (UHBD)[14] and are described in Ref. 4.

3 Results

	rate constant $\pm$ error $[10^{10}\frac{1}{Ms}]$	
I[mM]	neamine	model compound
150	2.42 ± 0.03	1.87 ± 0.03
200	2.22 ± 0.04	1.67 ± 0.03
250	2.10 ± 0.04	1.48 ± 0.03
300	1.86 ± 0.04	1.25 ± 0.03

Table 1. Association rate constants and their dependence on ionic strength derived from BD simulations. Error values are estimated at the 90% confidence level.

Rates of association and their dependence on ionic strength are shown in Table 1. Neamine, which is smaller than its derivative diffuses faster. For both antibiotics a rather weak dependence of computed association rates on ionic strength is observed. The decrease in association rates upon change of ionic strength is about 23% for neamine and about 33% for its proposed derivative. Both molecules bear the same net total charge and the observed difference arises from different distributions of partial charges (as the total charge of neamine is distributed in smaller volume).

In our previous study[4], we represented neamine with a much simpler, two-bead model. The observed decrease in association rate upon changing ionic strength from 150mM to 300mM was about 5% and computed association rates had slightly higher values. A more detailed 13-bead model used in the present study probably better reproduces the effects arising from the distribution of partial charges, thus larger changes in neamine's association rates with ionic strength. Nevertheless, the influence of ionic strength on the binding kinetics is rather insignificant in both cases.

Previously, we observed that aminoglycosidic antibiotics slide along the RNA groove prior to binding. Simulations with detailed antibiotic models for neamine and its derivative reveal similar behavior.

4 Conclusions

We applied BD to determine the association rate constants of neamine and its proposed derivative. The compounds were modeled with an increased number of beads in comparison to our previous study[4]. Results obtained by describing aminoglycosides with just a few and over 10 beads showed comparable mechanisms of the encounter complex formation, similar magnitude of association rate constants but stronger dependence on ionic strength with a more detailed description of antibiotic.

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